High-Throughput Tracking of Pluripotent Human Embryonic Stem Cells with Dual Fluorescence Resonance Energy Transfer Molecular Beacons.

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Public Summary:

Scientific Abstract:
Pluripotent human embryonic stem cells (hESCs) provide an unprecedented opportunity for the study of human tissue development, and the development of cell-based therapies for human disease. To realize these potential advances, however, methods for monitoring expression of intracellular proteins in live hESCs without altering cellular properties are needed. Molecular beacons are single-stranded oligonucleotides that have been employed to assay gene expression. To test their potential for high-throughput isolation of hESCs, we developed a dual fluorescence resonance energy transfer (FRET) molecular beacon system using fluorescence-activated cell sorting (FACS) with Oct4 as a target. We demonstrate that Oct4 can be detected by FRET using confocal microscopy, that this can be applied in a high-throughput manner to the identification and isolation of Oct4-expressing hESCs by FACS, that FRET-positive hESCs demonstrate pluripotency in culture and in vivo, and that hESCs transfected with molecular beacons demonstrate normal growth rates and oligonucleotide extinction over time. These studies demonstrate that FRET-based FACS using molecular beacons provides a useful tool for isolating Oct4-expressing pluripotent hESCs, and may also be adapted to selecting differentiating hESCs at specific developmental time points determined by transcription factor expression without functional or genomic alteration. As such, it provides an important new method for high-throughput isolation of hESC-derived tissue-specific precursors for analytic and therapeutic purposes.